THE BREEDING SYSTEM AND EFFECTIVENESS OF INTRODUCED AND NATIVE POLLINATORS OF THE ENDANGERED TROPICAL TREE Goetzea elegans (Solanaceae)

Marcos A. Caraballo-Ortiz1,2,3* and Eugenio Santiago-Valentín1,2,3

1Department of Biology, University of Puerto Rico, Rio Piedras Campus, P.O. Box 70377, San Juan, Puerto Rico 00936–8377, USA
2Herbarium, Botanical Garden of the University of Puerto Rico, 1187 Calle Flamboyán, San Juan, Puerto Rico 00926, USA
3Center for Applied Tropical Ecology and Conservation, University of Puerto Rico, Rio Piedras Campus, P.O. Box 23341, San Juan, Puerto Rico 00931–3341, USA

Abstract—The impact of introduced species on native organisms is one of the main conservation concerns around the world. To fully understand the effect of introduced pollinators on native plants, it is important to know the reproductive biology of the focal species, especially its pollination biology. In this study we examined the breeding system of the endangered tree Goetzea elegans (Solanaceae), and compared pollination effectiveness of the two main floral visitors, Coereba flaveola (an avian nectarivore), and Apis mellifera (the introduced European Honeybee). We assessed the breeding system of G. elegans by applying several pollination treatments to flowers of cultivated trees to test fruit set, seed set, and seed viability. We also examined the pollination efficiency of A. mellifera and C. flaveola, and compared all the treatments with positive and negative controls. Our results indicate that the introduced honeybee A. mellifera is as efficient as the native bird C. flaveola in pollinating the flowers of G. elegans. This study also showed that G. elegans requires cross-pollination for fruit and seed set, and to obtain high seed viability rates. Despite the fact that many studies report exotic species as detrimental for native organisms, we document a case where an introduced insect has a beneficial impact on the reproductive biology of an endangered tropical tree.

Key words: Apis mellifera, Coereba flaveola, geitonogamy, outcrossing, Puerto Rico, selfing.

INTRODUCTION

The negative impacts of introduced species on ecosystems are a major issue in conservation biology (e.g. Callaway et al. 2004; Lugo 2005; Nogueira-Filho et al. 2009; Davis et al. 2010; Nunez et al. 2010). Introduced species can disrupt native plant-animal interactions, such as plant-pollinator mutualisms, which can have negative effects on reproductive success (Traveset & Richardson 2006; Aizen et al. 2008; Bartomeus et al. 2008; Padrón et al. 2009). One of the most common introduced pollinators around the world is the honeybee, Apis mellifera L. (Apidae). Honeybees are native to Europe, Africa and the Middle East (Winston 1987), and they have been intentionally introduced in many countries to improve crop pollination and to produce honey (Hansen et al. 2002). The impacts of the introduction of A. mellifera on plant communities are a focal point of debate (Goulson 2003; Stanley et al. 2004; Moritz et al. 2005; Traveset & Richardson 2006; Kaiser-Bunbury & Müller 2009), with studies showing honeybees to be neutral or beneficial for the pollination of native plants (Gross 2001; Dupont et al. 2004; Fumero-Cabán & Meléndez-Ackerman 2007), especially for dispersing pollen in fragmented habitats (Dick 2001; Dick et al. 2003). Conversely, other studies on the pollination of tropical plants show that A. mellifera has negative impacts on the fitness of native plants (Goulson 1998; do Carmo et al. 2004), on interactions with native pollinators by displacing them (Kato et al. 1999; Hansen et al. 2002; Goulson 2003), and they tend to be less efficient pollinators than native species, including other bees and birds (Westerkamp 1991; Freitas & Paxton 1998; Gross & Mackay 1998; Hansen et al. 2002).

The ecological disruption of introduced species on plant-pollinator mutualisms is expected to be more severe for organisms restricted to islands because island populations are usually smaller, have limited dispersal, and are believed to be inferior competitors than continental populations (Cronk & Fuller 1995; Whittaker 1998). In addition, islands often have depauperate pollinator communities which provide services to a wide array of plants (Olesen et al. 2002). However, rare plant species are often less likely to receive pollinator visits due to competition with more abundant floral resources of surrounding common plant species (Rymer et al. 2005). Pollen limitation and the resultant low seed set are particularly detrimental for rare tropical trees, as most of the species in the tropics are dioecious or self-incompatible (Bawa et al. 1985; Ward et al. 2005). The combination of pollen...
limitation and habitat destruction of tropical forests is believed to be especially harmful for rare self-incompatible trees in biodiversity hotspots. This is because in biodiversity hotspots, intense competition for pollinator services and high deforestation rates are prevalent (Vamosi et al. 2006).

The objectives of this study were to examine the impact of the introduced *A. mellifera* on the pollination system of the tropical tree *Goetzea elegans*, an endemic and rare tree found in the Caribbean Biodiversity Hotspot (Fig. 1a; Myers et al. 2000), to compare the pollinator effectiveness of *G. elegans* floral visitors, and to examine the *G. elegans* breeding system. Specifically, we hypothesized that (1) *A. mellifera* pollinates *G. elegans* less efficiently than its native pollinator, as found in previous studies on the impact of *A. mellifera* on pollination of native plants (Westerkamp 1991; Freitas & Paxton 1998; Hansen et al. 2002). We also hypothesized that (2) *G. elegans* is self-incompatible and requires outcrossing to set seeds, as this is the predominant reproductive strategy in tropical trees (Bawa et al. 1985, Nason & Hamrick 1997, Ward et al. 2005).

**MATERIALS AND METHODS**

**Study species**

*Goetzea elegans* Wydler is an endangered tree of the subfamily Goetzoeideae in the Solanaceae (sensu Olmstead et al. 2008) and only found in populations on the northwestern and the eastern region of the island of Puerto Rico (including Vieques Island; USFWS 1987; IUCN 2010 [C2a version 2.3]). Although *G. elegans* was once considered critically endangered, totalling around 50 individuals in three populations (USFWS 1987), new populations have been recently discovered. The total population is now estimated at around 820 individuals in 12 populations, most of them fragmented and restricted to ravines. As for most of the species designated as endangered, very little is known of its life history, such as breeding system, pollinators, dispersal agents and demographic characteristics. Preliminary studies by Santiago-Valentín (1995) suggest that *G. elegans* requires cross pollen to set fruits. However, additional experiments are needed to confirm these preliminary data. Although the populations of *G. elegans* have declined mainly due to deforestation and land-use changes (USFWS 1987), they may also be suffering the impact of introduced species.

In the wild, *G. elegans* trees can reach up to 18 m in height, and produces flowers and fruits throughout the year, with peak flowering occurring between February and July (Little et al. 1974; Santiago-Valentín 1995). The flowers are perfect with no fragrance perceptible to humans. The pale yellow corolla is funnel shaped, up to 2 cm long and 1.3 to 2 cm across (Fig. 1a). Six slender stamens are borne near the base of the corolla and are exerted. The pistil has a slender style with a bilobed stigma, and a hairy 2-celled ovary bearing few ovules. The fruits are orange drupes about 4 cm long covered by velvety hairs with a persistent calyx (Little et al. 1974). Fruit shapes are variable among trees (from round to pear-shaped), with shape being consistent within one tree. Fruits are commonly one-seeded, but fruits with two and up to nine seeds (more rarely) have been observed in the wild (Santiago-Valentin 1995). The seeds are elliptic and about 0.7 cm in size (Little et al. 1974).

The flowers of *G. elegans* are visited by both birds and insects. The most frequent flower visitor is the native nectarivorous bananaquit *Coereba flaveola* (b), and receiving a visit from *Apis mellifera* (c). Photo credits: W. Hernández Aguiar (a), T. A. Carlo Joglar (b), and M. A. Caraballo Ortiz (c).

The flowers of *G. elegans* are visited by both birds and insects. The most frequent flower visitor is the native nectarivorous bananaquit *Coereba flaveola* Bryant (Coerebidae; Fig. 1b; USFWS 1987; Santiago-Valentín 1995), which is considered the most common bird in Puerto Rico (Raffaele et al. 1998). *Coereba flaveola* has a reputation
for robbing the nectar of many plant species (Kodric-Brown et al. 1984; Ricart 1992; Fumero-Cabán & Meléndez-Ackerman 2007), and although the culmen of *C. flaveola* appears to contact the reproductive structures of *G. elegans*, we lack experimental data confirming its role as a pollinator of this tree species. Another common visitor of *G. elegans* is the common honeybee *Apis mellifera* L. (Apidae; Fig. 1c; Santiago-Valentín 1995). This introduced bee has been observed foraging on flowers of many native species throughout the island in both managed and wild colonies. Both *C. flaveola* and *A. mellifera* are common visitors of *G. elegans* flowers in all of the wild populations of the species (M. A. Caraballo-Ortiz, pers. obs.). Aside from *C. flaveola* and *A. mellifera*, *G. elegans* flowers are infrequently visited by two native hummingbird species (*Anthracothorax dominicus aurulentus* Audebert & Vieillot and *Eulampis holosericeus holosericeus* L.; Santiago-Valentín 1995; M. A. Caraballo-Ortiz, pers. obs.). Although there are other common pollinator species in Puerto Rico (e.g. *Xylocopa* carpenter bees), they have not been observed interacting with *G. elegans* flowers.

**Study site**

Our study was conducted at the Botanical Garden of the University of Puerto Rico in San Juan, Puerto Rico (18°23′38.17″ N, 66°03′46.35″ W; 19 m asl), located within the subtropical moist forest zone (Holdridge system: Ewel & Whitmore 1973). All *G. elegans* plants used in this study were grown from seeds collected randomly from a wild population of about 50 trees at the municipality of Isabela, in northwestern Puerto Rico (Rafael Rivera-Martínez [Conservation Trust of Puerto Rico], pers. comm.). Experiments were performed from November 2003 to January 2005 with cultivated trees of *G. elegans* that had reached maturity. Under cultivation conditions, *G. elegans* begin first flowering during the first two or three years of age. Accessibility to an adequate number of trees and flower samples, as well as the fact that the two putative pollinators are common residents of the Botanical Garden, made the cultivated setting an ideal set-up for the pollination experiments.

**Pollination treatments**

We selected 20 cultivated adult trees of *G. elegans* to examine its breeding system. The trees were planted in 30 L pots, reaching a mean height of 2.5 m (S.E. ± 0.035 m) and all of them were positioned within an area of about 50 m². Each of the 20 experimental trees was subjected to 13 pollination treatments. Each pollination treatment was replicated three times (i.e. on three different flowers) on each tree (i.e. 39 flowers on each of the 20 trees; 780 flowers in total). The 13 pollination treatments were: (1) visits limited to *A. mellifera*, (2) visits limited to *C. flaveola*, (3) exposed flowers, (4) bagged flowers (see details of bagging technique below), (5) outcrossed hand pollination, (6) geitonogamous hand pollination, and (7) selfed (same flower) hand pollination. To exclude the possibility that experimental flowers were contaminated with their own pollen, treatments 1-6 were repeated with emasculated flowers (representing treatments 8-13, respectively). The following describes details of each treatment.

For the exposed pollination treatment, flowers were left open continuously to all visitors until abscission. In the bagged treatments, flowers were kept covered to exclude all pollinators. Flowers used to test pollination by animal vectors (*A. mellifera* and *C. flaveola*) were kept bagged and only exposed to allow a single visit per flower. Immediately after the vector visit, we re-bagged the flower and waited for fruit production. In the hand-pollinated treatments, flowers were bagged at all times except while performing manual pollinations and emasculations. For the outcrossing treatment, pollen was manually transferred between trees, and for the geitonogamy treatment, pollen was manually applied among flowers of the same tree. To examine within-flower selfing, pollen was manually transferred from anthers to the stigma of the same flower.

Hand-pollinations were performed using a fine brush with a long, pointed tip. The brush was rinsed thoroughly with water and dried between each pollen transfer to avoid contamination. Emasculations were performed one day prior to flower anthesis (and dehiscence of anthers) by removing all anthers in the flower bud with forceps. For all treatments involving bagged flowers, flower buds were covered one day prior to anthesis using a pollen cloth bag. Pollen is a lightweight, non-woven fabric used in the clothing industry that allows light and air to pass through. It has previously been used to cover flowers (Wyatt et al. 1992) without altering their development or longevity (Santiago-Valentín 1995). For each of the pollination treatments, fecundity was measured in three different ways: as fruit set, as seed set, and seed viability. Seedless fruits were classified as aborted and considered as zero in the fruit set analysis and not included in the seed set analysis. Seed viability tests were carried out at the Conservation Trust of Puerto Rico nurseries (located within the grounds of the Botanical Garden of the University of Puerto Rico in Rio Piedras, Puerto Rico) by germinating the seeds in a humid peat moss bed with low sunlight conditions (to simulate natural germinating conditions for this species) and verifying normal shoot and root growth from January 2004 to April 2005. All seeds collected from an individual tree were planted in a block, and all the blocks were positioned at random along the peat moss bed.

**Statistical analyses**

We tested for differences in fruit set, seed set and seed viability among the 13 pollination treatments first by using the fraction of fruit set as the response variable. Fractions were calculated on a per-flower basis (N = 3 flowers per treatment per tree, thus, possible outcomes were 0, 0.33, 0.66 and 1). Seed set data were normalized using a logarithmic transformation, while seed viability data were normalized using an arcsine transformation. In statistical analyses, trees (N = 20 individuals) were treated as blocks where all treatments were represented and replicated. First we analyzed fecundity measures altogether using a multivariate analysis of variance (MANOVA; SAS Institute 2000) given that we measured multiple and correlated response variables on the same treatments. In the MANOVA, the responses were the percentage of fruit set per flower, seed number per flower and, percentage of seed viability per flower, while the factors were pollination treatments, individual, and the interaction between...
pollination treatments and individual. Following significance of this analysis (and as recommended by Scheiner 2001), we conducted post hoc univariate tests using two-way ANOVA to test the effects of pollination treatments (at 13 levels), individual, and the interaction terms using JMPIN (SAS Institute 2000). In the case of fruit set, we used a Kruskall-Wallis ANOVA (and post hoc pairwise Wilcoxon Signed Rank tests with Bonferroni correction) given that data structure violated ANOVA assumptions. Results are presented as mean ± standard deviation.

RESULTS

Pollinator visits

Visits by *A. mellifera* produced fruit on 28% of the unemasculated flowers, and 35% of the emasculated flowers (Fig. 2a). The average number of seeds recorded for *A. mellifera* visits was 0.7 ± 0.2 seeds in the unemasculated flowers, and 0.9 ± 0.2 seeds in the emasculated flowers (Fig. 2b). Lastly, seed viability in the unemasculated and in the emasculated flowers was 12% and 22%, respectively (Fig. 2c).

Following a similar pattern to *A. mellifera*, visits by *C. flaveola* produced fruit on 22% of the unemasculated flowers and 18% of the emasculated flowers (Fig. 2a). Seed set and seed viability for the unemasculated flowers visited by *C. flaveola* were 0.5 ± 0.2 seeds and 10% respectively, whereas the values for the emasculated flowers were 0.6 ± 0.2 seeds and 15%, respectively (Fig. 2b, c).

Although *A. mellifera* and *C. flaveola* were effective pollinators of *G. elegans*, in contrast to our expectations, both animals were statistically similar for all of the fecundity measures (Fig. 2). Also, we did not detect statistical differences between any of the unemasculated and emasculated pollination treatments for either *A. mellifera* and *C. flaveola* (Fig. 2).

Breeding system

The unemasculated exposed flowers showed 50% fruit set, while the emasculated exposed flowers yield a fruit set of 47% (Fig. 2a). For seed set and seed viability, the values for the exposed unemasculated flowers were 1.7 ± 0.3 seeds and 32% respectively, and for the emasculated flowers were 1.8 ± 0.3 seeds and 26%, respectively (Fig. 2b, c). On the other hand, the bagged treatment produced the lowest values for fruit set, with 3% for unemasculated flowers and 0% for emasculated flowers (Fig. 2a). Additionally, the unemasculated bagged flowers had a seed set of 0.2 ± 0.1 seeds, and a seed viability of 3% (Fig. 2b, c).

The highest fruit yield (70%) was recorded in the unemasculated outcrossed flowers, followed by its emasculated counterpart (57%; Fig. 2a). Similarly, the outcrossed pollination treatment obtained the highest seed set and seed viability for the unemasculated (2.7 ± 0.3 seeds and 52% viability) and for the emasculated (2.2 ± 0.4 seeds and 34% viability) flowers when compared to the other pollination treatments (Fig. 2b, c). In sharp contrast to the outcrossed treatment, the selfed treatment produced a fruit set of 7% and the lowest values for seed set and for seed viability, with 0.1 ± 0.7 seeds and 2% viability (Fig. 2). The geitonogamy pollination treatment followed the same pattern as the selfed treatment, with a fruit set of 8%, a seed set of 0.2 ± 0.1 seeds, and a seed viability of 5% in the unemasculated flowers (Fig. 2). Likewise, emasculated flowers in the geitonogamy treatment produced a fruit set of 17%, a seed set of 0.4 ± 0.1 seeds, and a seed viability of 4% (Fig. 2).

While the bagged, selfed and geitonogamy treatments were not significantly different from each other, they were different from the exposed and outcrossed pollination treatments for all fecundity measures. The fruit set of the pollinator visitations was significantly different from the bagged, selfed and geitonogamy treatments (except for the emasculated geitonogamy treatment; \( X^2 = 178, df = 12, P = 0.0001 \); however, seed set and seed viability values were not significantly different among pollination treatments (Fig. 2). Moreover, we did not detect significant differences among any of the unemasculated and emasculated pollination treatments for the breeding system (Fig. 2). The MANOVA test detected significant differences among the pollination treatments, the individual *G. elegans* tree, and the interaction

![Fig. 2](image-url)

**Fig. 2.** Pollination treatments among unemasculated and emasculated flowers of *Goetzea elegans*. Columns show standard error bars, and different letters indicate statistical differences among treatments (\( \alpha = 0.05 \)). Pollination treatments include visits of *Apis mellifera* (*Aps*), visits of *Coereba flaveola* (*Coer*), exposed flowers (*Exp.*), bagged flowers (*Bag.*), outcrossed pollination (*Out.*), geitonogamous pollination (*Geito.*), and selfed pollination (*Self.*). (a) Average percentage of fruit set (%). (b) Average number of seeds per flower. (c) Average percentage of seed viability (%).
between pollination treatments and individual (Tab. 1). In the model, the pollination treatments were the most important factor explaining the variance (46%), followed by the individual (8%) and the interaction between pollination treatments and the individual (3%). After performing the two-way ANOVA tests, we found significant effects of pollination treatments ($F_{1,228,520} = 22.5, P < 0.0001$), individual ($F_{1,228,520} = 3.9, P < 0.0001$), and the interaction between pollination treatments and individual ($F_{228,520} = 1.4, P = 0.002$) on the seed set. Similarly, for the seed viability tests, we found significant effects of pollination treatments ($F_{1,228,520} = 17.1, P < 0.0001$), individual ($F_{1,228,520} = 3.1, P < 0.0001$), and the interaction between pollination treatments and individual ($F_{228,520} = 1.4, P = 0.001$).

Table 1. Results for the multivariate analysis of variance (MANOVA) for the whole model, for pollination treatments, for individual trees of *Goetzea elegans*, and for the interaction between pollination treatments and individual trees.

<table>
<thead>
<tr>
<th>Source</th>
<th>Pillai’s trace (F)</th>
<th>df</th>
<th>(P)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Whole model ((R^2 = 0.57))</td>
<td>0.93</td>
<td>1.75</td>
<td>518, 1040</td>
</tr>
<tr>
<td>Pollination Treatments</td>
<td>0.31</td>
<td>7.88</td>
<td>24, 1040</td>
</tr>
<tr>
<td>Individual Tree</td>
<td>0.19</td>
<td>2.81</td>
<td>38, 1040</td>
</tr>
<tr>
<td>Pollination Treatments x Individual Tree</td>
<td>0.75</td>
<td>1.36</td>
<td>456, 1040</td>
</tr>
</tbody>
</table>

**DISCUSSION**

We found that both *A. mellifera* and *C. flaveola* were legitimate pollinators of *G. elegans*, with similar pollination effectiveness per visit. In addition, *G. elegans* is partially self-incompatible, requiring pollen outcrossing to produce a high fruit set. Outcrossing is the most common breeding system in tropical trees, although there are varying degrees of self-compatibility (Bawa et al. 1985; Doligez & Joly 1997; Nason & Hamrick 1997; Dick et al. 2003; Ward et al. 2005).

**Pollinator visits**

The legitimate pollination of *G. elegans* by *A. mellifera* corroborates results from some studies showing the beneficial role of the introduced honeybee as a pollinator of native plant species (Vaughton 1992; Dick 2001; Gross 2001; Dick et al. 2003; Fumero-Cabán & Meléndez-Ackerman 2007), and differs from those where *A. mellifera* has been found to be less effective than native nectarivorous birds (Vaughton 1996; Hansen et al. 2002). However, our results support the idea that the effectiveness of *A. mellifera* as pollinator is contingent on the structural attributes (size and shape) of flowers, as well as on the foraging behaviour of *A. mellifera* (Vaughton 1996). For example, when *A. mellifera* visited *G. elegans* flowers to collect pollen, it landed and crawled throughout the anthers and usually made contact with the nearby stigma, thus promoting pollination (Fig. 1b).

Conversely, when *A. mellifera* foraged for nectar, it landed on the inner surface of the corolla lobes from which it inserted its proboscis into the nectaries (located at the base of the ovary), which did not usually result in pollination (M. A. Caraballo-Ortiz, unpubl. data).

The pollination role of *C. flaveola* in *G. elegans* contrasts with their robbing behaviour reported in several studies on flower visitation (Kodric-Brown et al. 1984; Askins et al. 1987; Fumero-Cabán & Meléndez-Ackerman 2007). Ricart (1992) determined that robbing or legitimate pollination behaviour of *C. flaveola* was dependent on corolla length. The short size of the corolla tube in *G. elegans* (11.1 ± 1.6 mm in length and 10.2 ± 1.6 mm in width) provides easy access for *C. flaveola* to nectar (culmen length = 13.2 ± 0.7 mm), and the exerted stamens promote pollen deposition on the bird’s forehead and consequently, pollen transport. Robbing by *C. flaveola* has been reported only in other plant species bearing longer corolla tubes (> 19 mm in length, unpublished data), where legitimate access to the flower nectaries by those birds with short bills is prevented by the disparity between bill and corolla length.

**Breeding system**

We detected a strong effect of inbreeding depression in the seed viability of the selfed treatments, suggesting that a single generation of selfing is sufficient to lower the germination vigour of a mainly outcrossing species such as *G. elegans*. By being partially self-incompatible, *G. elegans* decreases the deleterious effects of inbreeding and promotes genetic diversity in its populations (Charlesworth & Charlesworth 1979). Outcrossing plants generally have higher recombination rates, and thus, are more genetically variable and have higher levels of heterozygosity within populations than selfing plants (Hamrick et al. 1979; Loveless & Hamrick 1984; Hamrick & Godt 1989). Recombination is particularly important for plants that exist in small populations, as they often suffer from lower genetic diversity due to founder effects (Pflosser et al. 2005). Although outcrossing assures genetic variability of populations, a degree of self-compatibility enables reproduction in isolation when cross pollen limits seed set. In the Solanaceae family, there are several mechanisms through which the self-incompatibility system of a species can allow self-fertilization. These include changes in environmental conditions, the age of the flowers, and mutations in the S-alleles, among others (e.g., Levin 1996; Tsukamoto et al. 2003; Travers et al. 2004). It is possible that the partial self-compatibility in *G. elegans* evolved as a mutation that became fixed in the population, due to the fact that it increases the probability of reproduction in isolation or in fragmented habitats. As with *G. elegans*, other plants in Solanaceae have been found with partial self-incompatibility, such as the weed *Solanum carolinense* L., which has a plastic self-incompatible system (Travers et al. 2004).

The low fecundity exhibited by single visits of both *C. flaveola* and *A. mellifera* when compared to the exposed pollination treatment suggests that repeated visits to the flowers of *G. elegans*, which remained open an average of 3.4 days (S.D. ± 0.82), are necessary to increase chances of pollen deposition. However, the outcrossed pollination treatment
achieved higher fecundity rates than the exposed treatment, indicating that a significant proportion of pollen deposition in exposed flowers might be the result of either heterospecific pollen or geitonogamy. Cultivated *G. elegans* trees used in this study usually displayed several dozens of flowers at once, which increases the probability of geitonogamous pollination. We predict that geitonogamy is frequent in larger and isolated wild trees, where thousands of flowers are presented to visitors at once.

The statistical similarity between the emasculated and unemasculated pollination treatments suggests that the pollen present in mature anthers does not interfere (i.e. blocking or clogging) with pollen deposition on the stigma, or that outcross pollen is more vigorous and fertilizes the ovaries faster than selfed pollen (Cruzan 1989; Aizen et al. 1990; Snow & Spira 1993). The fecundity of unemasculated bagged flowers was similar to the selfed treatment, demonstrating the ability of *G. elegans* flowers to set fruit in the absence of pollinators (i.e., autogamy). Autogamy was facilitated by the proximity of the anthers to the stigma due to the similar length of the reproductive whorls in most of the examined flowers. We observed, however, that a few trees presented a degree of herkogamy. Herkogamy - a disparity in the length of stamens and style of flowers - prevented those *G. elegans* trees from performing autogamy, and could be interpreted as an additional mechanism to promote outcrossing (Motten & Antonovics 1992). Finally, the lack of fruits in the emasculated bagged treatment suggests that *G. elegans* does not produce seeds by agamospermy, thus it requires pollen for sexual reproduction.

**Conclusions**

Unlike many introduced species, *A. mellifera* has a positive effect on the fecundity of the tropical endangered tree *G. elegans*. Besides being an additional pollinator, the ability of *A. mellifera* to connect isolated populations through pollen outcrossing in fragmented landscapes (Dick 2001; Dick et al. 2003) is of particular importance for rare and partially self-incompatible species such as *G. elegans*, since cross-pollination is critical for their reproduction. At present, the scattered distribution of *G. elegans* trees in the wild and the considerable geographic distance among populations (mean: 36.1 Km ± 55.3) could prevent optimal outcrossing, thus jeopardizing the continuous genetic diversity of the species. Currently, the main threats to *G. elegans* survival are habitat destruction and modification, such as deforestation for agriculture, selective logging for fence posts, and limestone quarrying, as these activities promote fragmentation of populations, decline of reproductive trees, and reproductive isolation of remnant trees (USEFS 1987). Although nearly all *G. elegans* populations are restricted to small canyons or ravines, which also shelter important populations of other rare and endangered plants and animals, none of these areas have legal designation for protection. However, propagation efforts and establishment of ex situ populations in protected areas has already been initiated (Rafael Rivera-Martínez [Conservation Trust of Puerto Rico], pers. comm.). The establishment of these ex situ populations, in conjunction with the conservation of extant wild populations and the effective pollination of wild trees by *C. flaveola* and *A. mellifera* are essential to maintain viable populations and to promote the conservation of *G. elegans*.

**ACKNOWLEDGMENTS**

The authors thank J. D. Ackerman, J. M. Wunderle, T. Giray, T. A. Carlo, R. Tremblay, E. Meléndez-Ackerman, C. Trejo, J. E. Aukema, A. W. Norris, R. Glenn, S. Yang, and A. Stephenson for their insightful comments and revisions on the manuscript. We also thank S. Flecha, W. Caraballo Castraing, L. Ortiz Ortiz, W. Caraballo Ortiz, R. Rivera Martínez, J. Rodríguez, J. Soltzter, K. Umpierre, V. Manguel, S. Hernández, L. Rivera, E. Napoleón, C. Díaz, J. Colón, F. Ortiz, J. Rosario, C. Caló, B. Carrión, M. Miranda, N. M. Martínez, M. Robles, M. Vega, O. Díaz, A. Quiones, M. Gómez, N. Cortés, D. Cuba, G. Bone, R. L. González, D. Torres, C. Viera, E. Vega, B. Martínez, A. M. Camacho and R. Rivera for providing materials and assistance with experimental work. We are grateful to the Botanical Garden of the University of Puerto Rico and to the Conservation Trust of Puerto Rico for permitting research at their facilities. This research was funded by National Science Foundation-Centers of Research Excellence in Science and Technology (CREST: HRD-0206200) through the Center for Applied Tropical Ecology and Conservation of the University of Puerto Rico (CATEG). This is scientific contribution number 002 of the Herbarium of the University of Puerto Rico Botanical Garden (UPR).

**REFERENCES**


Montz RFA, Hartel S, Neumann P (2005) Global invasions of the western honeybee (Apis mellifera) and the consequences for biodiversity. Ecoscience 12:289–301


